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Reduced carnitine palmitoyl transferase activity and altered acyl-trafficking in red blood cells from hemodialysis patients

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Abstract

We measured carnitine palmitoyl transferase activity, free carnitine, and long chain acyl carnitine levels in erythrocytes from 15 uremic patients and 25 controls. Carnitine palmitoyl transferase levels in patients were significantly lower than in controls. The levels of free carnitine and long chain acyl carnitines as well as the long chain acyl carnitine/free carnitine ratio were significantly higher in patients than in controls. Our results suggest that hemodialysis causes alteration in the acyl-trafficking in red blood cells membrane.

Keywords: Carnitine palmitoyl transferase; Red blood cell; Carnitine; Membrane phospholipid turnover; Hemodialysis

1. Introduction

Erythrocyte carnitine palmitoyl transferase (CPT) is involved in the membrane phospholipid (PL) fatty acid turnover of human red blood cells (RBC) [1–3]. The reversible transfer of long chain fatty acids from CoA to L-carnitine, catalyzed by RBC CPT, modulates the acyl CoA/free CoA ratio, which is critical for the regulation of the membrane PL fatty acid turnover [1]. Long chain acyl carnitines (LCAC) serve as a reservoir of acyl-moieties for the reacylation of membrane PL, which is catalyzed by lysophospholipid acyl-CoA transferase (LAT) [2].

Human erythrocytes have been shown to have significant amounts of L-carnitine and its esters [4,5]. Because RBC carnitines do not freely exchange with plasma, their presence in mature erythrocytes seems to stem from the reminiscent function of erythrocyte precursors [4]. RBC carnitine levels seem to regulate the activities of some enzymes of human erythrocyte membranes, as well as their molecular properties such as stability and rigidity [6].

In this work, we studied CPT and carnitine levels in RBC from both normal subjects and hemodialysis patients.

2. Materials and methods

2.1. Materials

L-Methyl-^{3}[H]carnitine hydrochloride (60 Ci/mmol) was obtained from Amersham Iberica (Madrid), [1-^{14}C]acetyl-CoA (40 mCi/mmol) was from ICN Radiochemical (Irvine, USA). Carnitine acetyltransferase and phenyl-methane-sulphonyl-fluoride (PMSF) were purchased from Boehringer Mannheim, FRG. Palmitoyl CoA was from Sigma Chemical CO (St. Louis MO, USA). All other reactives used were reagent grade.

2.2. Subjects

Blood samples were drawn from 15 chronically uremic patients (7 female, 8 male; aged 55.7 ± 11.4, range 40–72 years) undergoing regular hemodialysis treatment during 13 years. Also, blood samples were taken from 25 age-matched healthy controls (13 male, 12 female; aged 56 ± 10, range 42–70 years).

Abbreviations: CPT, carnitine palmitoyl transferase; PL, phospholipid; RBC, erythrocytes; LCAC, long-chain acyl carnitines; LAT, lysophospholipid acyl-CoA transferase; FC, free carnitine; COX, cytochrome-*c* oxidase.

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2.3. Preparation of washed RBC and ghosts

Venous blood was collected before dialysis, in the morning, and after fasting in heparin or EDTA. Leukocytes and platelets were removed by passage through a column of α-cellulose and microcrystalline cellulose [7]. RBC were then washed three times with cold 0.9% NaCl.

Membrane ghosts were prepared from washed heparinized cells which were lysed in 30 Vols. of cold lysing buffer (5 mM NaH_2PO_4, pH 7.4, 0.1 mM PMSF). The lysed cells were then centrifuged for 10 min at 30 000 × *g* at 4°C, and the resulting pelleted ghosts were washed four times. The ghosts were collected then in 5 mM NaH_2 PO_4 (pH 7.4). Protein concentration was determined according to Bradford [8].

2.4. CPT assay

The activity was measured in ghosts RBC by a radiochemical procedure [9] in 220 mM sucrose/40 mM KCl/10 mM Tris/HCl (pH 7.4 at 30°C)/1 mM EGTA, except that the final concentration of palmitoyl CoA was 50 μM, with 1.3 mg of BSA/ml; L-[^{3}H]carnitine was 1 mM, and the time of incubation was 30 min.

2.5. LCAC and FC determinations

LCAC and FC levels were measured by an optimized radiochemical enzymatic assay [10] in washed RBC collected in EDTA. Modifications were done as previously described [11].

2.6. Cytochrome-c oxidase (COX) activity

The activity was determined in ghosts RBC, by monitoring the decrease in absorbance at 550 nm of reduced cytochrome *c* [12]. Reduced cytochrome *c* was prepared fresh before each experiment by adding a few grains of sodium hydrosulfide to a 1% solution in 10 mM K-Phosphate buffer (pH 7.0)

2.7. Statistical analysis

Statistical analysis was performed by Student's *t*-test.

3. Results and discussion

Membrane PL fatty acid turnover in human RBC depends on the activities of both long chain acyl CoA synthetase and LAT [2]. The former generates acyl CoA from free fatty acids and CoA, while the latter reacylates lysophospholipids by using acyl CoA as substrate. When the rate of formation of acylCoA becomes different from that of its utilization for reacylation, acyl CoA pool and the acylCoA/free CoA ratio become altered, leading to disturbances in RBC PL fatty acid turnover [1]. RBC CPT maintains a favorable acyl CoA/free CoA ratio by forming acylcarnitine from carnitine and acyl CoA [1].

We found significantly reduced CPT levels in RBC from patients on HD compared with those of controls (Table 1). To assess whether the total CPT activity measured in RBC membrane preparations was contaminated with CPT activity from reticulocyte mitochondria, we determined the activity of cytochrome-*c* oxidase (COX), a mitochondrial membrane marker, in membrane preparations from 6 patients and 5 controls [1]. Activities of COX were very poor and similar in both controls (0.55 ± 0.08 nmol/min/mg protein; mean ± S.D.) and patients (0.57 ± 0.05 nmol/min/mg protein; mean ± S.D.). These data clearly indicate that the activity of CPT in membrane preparations is mostly contributed by RBC membranes, and that there is scant contamination with reticulocyte mitochondria.

The decrease in RBC CPT levels may cause similar consequences to those observed after CPT inhibition by 2-tetradecylglycidic or palmitoyl-D-carnitine [1]. Under such circumstances, the lack of buffering activity of CPT alters the acylCoA/free CoA ratio, and eventually the availability of fatty acid moieties for reacylation. Additionally, the relatively high concentrations of free CoA may further act as an inhibitor of the enzyme LAT. We suggest that the reduction in RBC CPT activity alters erythrocyte PL turnover, impeding the repairing of fatty acids damaged by free radicals, and ultimately leading to accumulation of lysophospholipids. The latter may be involved in the anemia and hemolysis from uremic patients.

In controls, we found levels of RBC carnitines similar to those previously documented [4,5]. In hemodialysis patients, RBC FC and LCAC values did not differ from

Table 1
Levels of FC, LCAC, CPT and LCAC/FC ratio in red blood cells from HD patients and controls

	FC	*LCAC*	*LCAC/FC*	*CPT*
Controls ($n = 25$)	58.5 ± 21.1	8.53 ± 2.81	0.15 ± 0.04	0.42 ± 0.14
HD Patients ($n = 15$)	103.5 ± 32.2 [b]	19.9 ± 8.48 [b]	0.20 ± 0.06 [a]	0.32 ± 0.17 [a]

FC, Free carnitine; LCAC, long chain acyl-carnitine; CPT, carnitine palmitoyl transferase.
FC and LCAC are in nmol · gHb^{-1}, and CPT is in nmol · min^{-1} · mg $protein^{-1}$. Data indicated are mean ± S.D.
[a] Significant differences vs controls ($P<0.05$; *t*-test).
[b] Significant differences vs controls ($P<0.001$; *t*-test).

those reported by Wanner et al. [5]. The levels of both FC and LCAC were significantly higher in patients than in controls (Table 1). Moreover, the LCAC/FC ratio was significantly greater in patients than in controls. Erythrocyte CPT is known to have a freely reversible flux, the direction of which relies upon the mass action ratio of the substrates [1]. Because of the high concentrations of FC in RBC of hemodialysis patients, the equilibrium of residual CPT shifts more likely towards acylcarnitine than towards acyl CoA. The observation that the proportion of LCAC to FC increases in RBC from hemodialysis patients strongly suggests this possibility. Alternatively, the difference in the LCAC/FC ratio could result from decreased use of fatty acyl groups or increased production. High levels of LCAC are known to inhibit RBC Na^+-K^+-ATPase [13]. In addition, molecular dynamics of human membrane [14], its stability, as well as its rigidity [6] seem to depend on RBC LCAC levels and on the ratio of LCAC to FC. Taken together, all these data support that high levels of LCAC and increased proportion of LCAC to FC in RBC may contribute to the pathogenesis of the anemia in hemodialysis patients.

In conclusion, we suggest that decreased CPT levels in RBC from HD patients alter acyl-trafficking in membrane phospholipid fatty acid turnover. The increase in RBC LCAC, and particularly in the LCAC/FC ratio, may account for, at least in part, some RBC abnormalities in hemodialysis patients.

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